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☐ 1: Biochemistry. 2000 Sep 5;39(35):10627-33.

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Specific binding of alpha-macroglobulin to complement-type repeat CR4 of the low-density lipoprotein receptor-related protein.

Andersen OM, Christensen PA, Christensen LL, Jacobsen C, Moestrup SK, Etzerodt M, Thogersen HC.

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The low-density lipoprotein receptor-related protein (LRP) is a large surface receptor that mediates binding and internalization of a large number of structurally and functionally unrelated ligands. The ligand binding sites are located in clusters of complement-type repeats (CR), where the general absence of mutual binding competition suggests that different ligands map to distinct sites. Binding of alpha(2)-macroglobulin-protease complexes to the LRP is mediated by the receptor binding domain (RBD) of alpha(2)-macroglobulin (alpha(2)M). To determine the major binding epitope(s) in the LRP, we generated a complete set of tandem CR proteins spanning the second cluster of CR domains, and identified a binding site for alpha(2)M in the N-terminal part of the cluster comprising CR3-CR6, using ligand blotting and surface plasmon resonance (SPR) analysis. The specific site involved in alpha(2)M recognition resides in the fourth CR domain, CR4, whereas another site is identified in CR5. An acidic epitope in CR4 is identified as important for binding alpha(2)M by mutagenesis and SPR analysis. The formation of the complex between the rat alpha(1)-macroglobulin RBD and CR domain pairs is characterized by analytical size-exclusion chromatography, which demonstrates a sufficiently strong interaction between the alpha(1)M RBD and CR34 or CR45 for the isolation of a complex.

PMID: 10978145 [PubMed - indexed for MEDLINE]

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